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A NEW CELL LINE ESTABLISHED FROM CALF KIDNEY

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Abstract: A new cell line "CK cell line" capable of continuous propagation was established from a calf kidney tissue. The bovine adenovirus type 3 could propagate well in this cell line.

Bovine adenovirus type 3 can induce undifferentiated tumors in adult hamsters as well as in newborn hamsters(1, 2). Since the tumor has characteristic features in tumor morphology and growth pattern, bovine adenovirus type 3-tumorigenesis needs to be studied in greater details. Cultured cell line, which is susceptible to this virus and is available at any time, is required for these studies. The authors have succeeded in establishing a calf kidney cell line for such a purpose.

The present paper deals with the history of the cell line and some of its properties.

MATERIALS AND METHODS

Medium: Nutrient medium used for the primary and serial cultures was Eagle's minimum essential medium (MEM) supplemented with 20 per cent fetal bovine serum.

Preparation of Primary Tissue Culture: A calf kidney was swabbed with 70 per cent alcohol and decapsulated. The kidney tissue was minced with scissors and washed twice with phosphate buffered saline (PBS). About 1 gm of minced tissue was suspended in about 100 ml of the solution containing an equal volume of 0.25 per cent trypsin and 0.5 per cent pancreatin, and stirred slowly at 4°C overnight for digestion. On the following day, cells were collected by centrifugation at 1,000 rpm for 5 min, suspended in the growth medium, divided into bottles, stoppered and cultured at 37°C.

Subculture: Bottles with vigorously growing cells were selected and used for further passages. Usually, three bottles were used for tissue cultures; two for a source of cell supply in continuous cultures and one kept in reserve. Nutient fluid was discarded, and cultured cells were quickly rinsed with PBS and treated with 0.1 per cent trypsin. Cells were collected by centrifugation

at 1,000 rpm for 5 min., resuspended in a 30 ml fresh nutrient medium and divided into three bottles. The cultures were incubated at 37°C for 3~7 days for sufficient cell growth. The nutrient medium was changed on the third or fourth day.

Growth Curve: The number of cells was counted by counting the numbers of cell nuclei according to the citric acid crystal violet method.

Chromosome Analysis: The air-dried chromosome preparations were made according to the air-drying technique of K. H. ROTHFELS and L. SIMINOVITCH (3) with some modification. The chromosome numbers were determined by counting 50 cells in metaphases on microscopic photographs taken at a high magnification.

Titration of Bovine Adenovirus Type 3: Bovine adenovirus type 3, WBR-1 strain, was titrated in the newly established calf kidney cell line and in the second subculture of calf kidney cell culture, and the titers in both cells were compared. The virus was diluted in ten-fold series and 0.1 ml of each dilution was inoculated into cell tubes with 1 ml maintenance medium. The maintenance medium consisted of MEM with 2 per cent calf serum. The inoculated tubes were incubated at 37°C for 21 days. The recording of titer was made on the seventh, 14th and 21st day by observing viral cytopathic effect. The titer was calculated according to Reed-Muench method.

RESULTS

Course of Establishment: In the early subcultures, there were a large number of morphologically different cell types, but mainly fibroblast-like cells and epithelial cells. Later, fibroblast-like cells became prominent around the ninth or tenth subculture, which remained almost unchanged morphologically throughout subcultures. At present, these cells are propagating without any decline in the growth rate for 52 subcultures (for about 7 months), confirming the establishment of a new cell line. This newly established cell line was designated as "CK cell line".

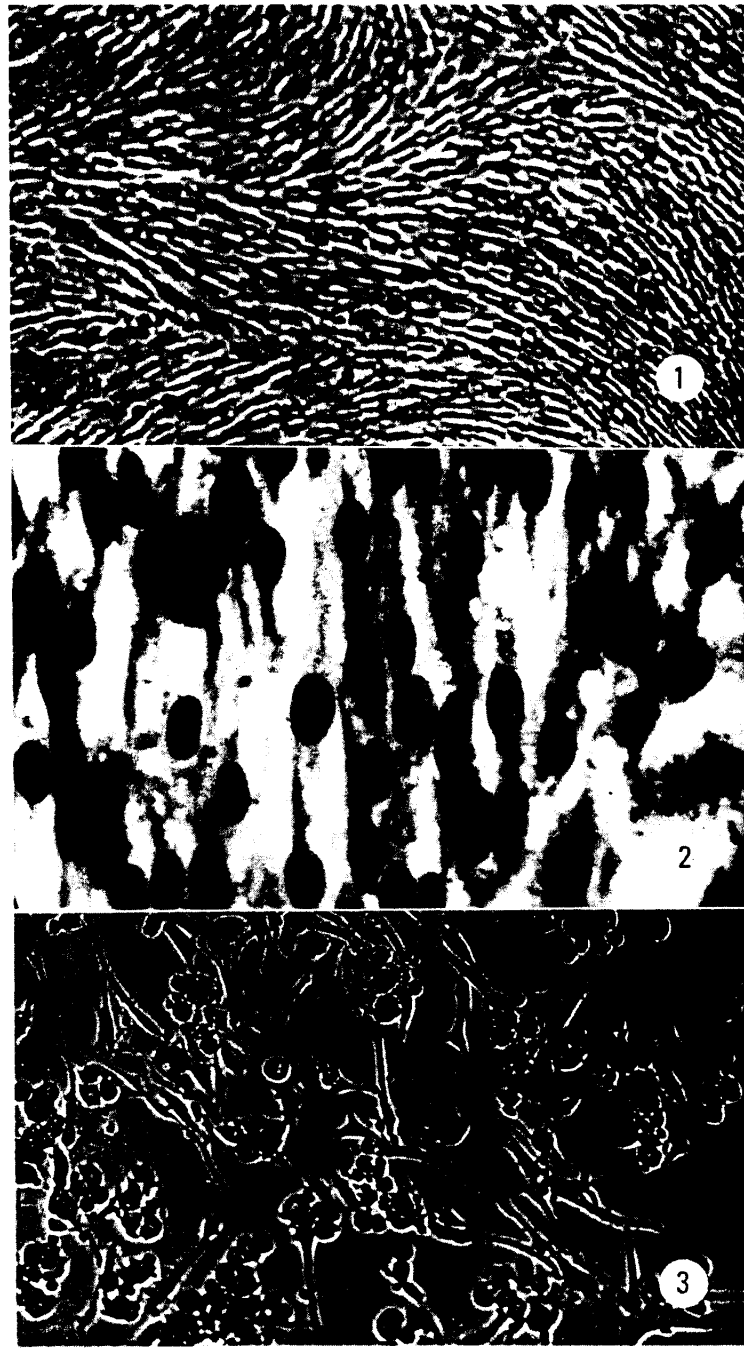
The cells propagated at the 33rd subculture are shown in Photo 1. The cells were spindle in shape, uniform in size and running in a parallel fashion. The nuclei were small, dark and oval in shape having a few nucleoli (Photos 1, 2).

Growth Properties: In the 33rd subculture, 5×10^4 cells were inoculated in a series of tubes containing 1.0 ml of the growth medium. An increase in the numbers of cell nuclei was calculated everyday. As show in Fig. 1, there was a lag phase of 44 hours before growth commenced and CK cells grew

Photo 1. Phase contrast figure of fibroblast-like cells in the 33rd subculture of "CK cell line", ×40.

Photo 2. Fibroblast-like cell in the 33rd subculture of "CK cell line", ×400, Hamatoxyline-eosin.

Photo 3. CK cells infected with bovine adenovirus type 3, showing cytopathic effect, ×100.


1
2
3

thereafter exponentially for four days, having a doubling time of 31.4 hours. After that, the growth rate gradually decreased with time, though complete cessation of the cell proliferation was not observed even on the seventh day.

Chromosome Analysis: At the 35th subculture, chromosome number in the CK cell line was examined. As shown in Fig. 2, chromosome numbers ranged in 52~121, 54 per cent of the metaphases being of normal diploid range (2n=60). Karyological analysis was not performed.

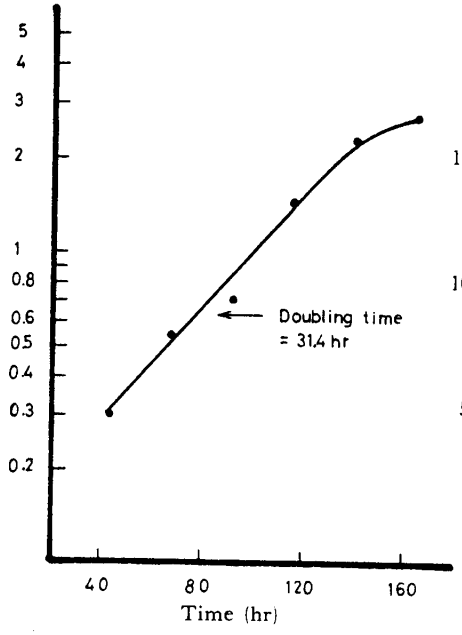
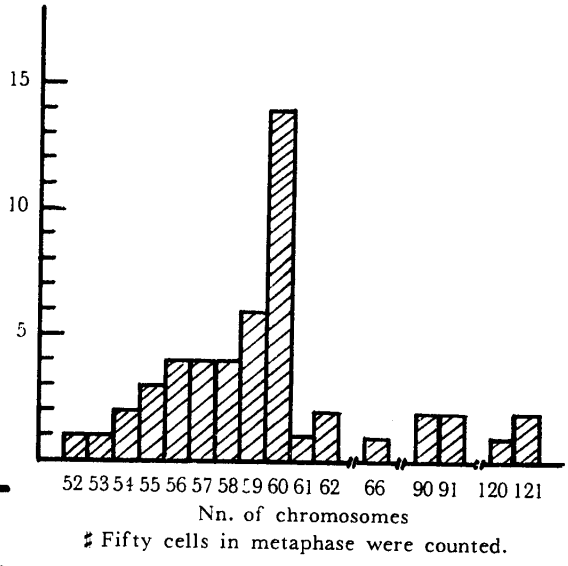


Fig. 1. A Growth curve at the 33rd subculture



* Fifty cells in metaphase were counted.

Fig. 2. Distribution of chromosome numbers at the 35th subcultue.

Susceptibility to Bovine Adenovirus Type 3: The bovine adenovirus type 3 propagated in the second subculture of calf kidney cell culture was titered in the second subculture of kidney cell culture and CK cells. The both cells inoculated with the virus revealed characteristic cell alterations; the cells became round, clustered and finally detached from the glass surface (Photo 3). The titer of the virus on the seventh, 14th and 21st day was $10^{2.5}$, $10^{3.5}$ and $10^{4.5}$ in the second subculture of calf kidney cells, and $10^{1.5}$, $10^{2.5}$ and $10^{3.5}$ $TCID_{50}$/0.1 ml in CK cells, respectively.

DISCUSSION

As the established cell line can be readily cultured and cells have rather stable properties, we conducted virus assay or quantitative experiments using

those cell lines possessing differential susceptibility to various viruses. Primary or secondary calf kidney cells have been selected as susceptive cells for bovine viruses including bovine adenovirus type 3, but these cells are not always available at given time and are in varying conditions in each occasion. Therefore, there is is still considerable need for established cell lines of calf kidney cells for various studies when using bovine viruses.

The literature contains hardly any references to established cell lines derived from calf kidney tissues. The cell line (MDBK) established by MADIN and DARBY (4) consists of fibroblast-like cells and supports the *in vitro* growth of vesicular stomatitis virus types Indiana and New Jersey and infectious bovine rhinotracheitis virus. The calf kidney cell line (CKT) established by KIMURA was originally epithelial cell (5). CKT cells are not susceptible to polio-virus types 1, 2 and 3 or to echovirus type 4, but they show a weak susceptibility to coxackie viruses B1 and B5. The KIMURA's cell line shows a susceptility similar to that of HeLa cells when challenged with adenoviruses (5). In addition, five cloned cells were derived from the CKT cells, and one clone CKT-1 had high susceptibility to bovine adenovirus type 3 (6).

On the other hand, the calf kidney cell line (CK) established by the authors has been propagating without decline of the growth rate for more than 50 subcultures, and they consist of fibroblast-like cells and are susceptible to bovine adenovirus type 3 in comparable degree to secondary culture of calf kidney cells. This cell line will be useful in veterinary virology.

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